

THE ROLES OF THE MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) PATHWAYS  
AND CELL WALL REMODELING IN RESPONSE TO ETHANOL STRESS IN  
SACCHAROMYCES CEREVISIAE

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M.Sc.(ENVIRONMENTAL BIOLOGY)

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ABSTRACT

During fermentation, yeast cells are often exposed to ethanol. Although the toxicity of ethanol to budding yeast *S. cerevisiae* has widely been studied, the ethanol stress signaling is still unknown. The growth of the mutants lacking genes involved in the mitogen-activated protein kinase (MAPK) pathway was examined under ethanol stress condition. Our results revealed that BCK1, MPK1, SWI4, and SWI6 involved in the cell wall integrity pathway, and STE3 and AKR1 involved in the pheromone signaling pathway, were required for tolerance to ethanol. In addition to the mutants of the cell wall integrity pathway, the  $\Delta ste3$  and  $\Delta akr1$  mutants were sensitive to a cell wall-perturbing agent, Calcoflour white, suggesting the role of these signaling molecules in maintaining the cell wall during ethanol stress. To investigate whether cell wall remodeling occurs in response to ethanol stress, Zymolyase sensitivity test was used to monitor the cell wall alterations. We found that ethanol induced cell wall remodeling in dose- and time-dependent manner. Furthermore, we also found that not only genes involved in cell wall integrity, but AKR1 is also important for cell wall remodeling during ethanol stress. Interestingly, the pretreatment of the  $\Delta bck1$  and  $\Delta mpk1$  mutants lacking signaling proteins of the cell wall integrity pathway with ethanol or Calcoflour white resulted in increased tolerance to Zymolyase, suggesting the importance of additional pathways in signaling cell wall stresses caused by ethanol and Calcoflour white. Our results of the gene expression revealed that the expression levels of FKS2, CHS3, and CRH1 were rapidly increased after the exposure to ethanol for 30 minutes. However, in the presence of Calcoflour white, the expression levels of these genes were induced after prolonged incubation for 4 hours. We further investigated the expression of the cell wall-related genes in the  $\Delta bck1$ ,  $\Delta mpk1$ ,  $\Delta swi4$ ,  $\Delta swi6$ ,  $\Delta ste3$ , and  $\Delta akr1$  mutants, and found that, in the  $\Delta bck1$  and  $\Delta mpk1$  mutants, the expression levels of cell wall-related genes, i.e. FKS2 and CRH1, were slightly increased after exposure to ethanol. These results suggested the role of alternative signaling pathways in response to ethanol when the cell wall integrity pathway is inactivated.

KEY WORDS: CELL WALL INTEGRITY PATHWAY/ ETHANOL/ MAPK PATHWAY/  
PHEROMONE SIGNALING PATHWAY/ *Saccharomyces cerevisiae*

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mitogen-activated protein kinase (MAPK)

*Saccharomyces cerevisiae*

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*Saccharomyces cerevisiae*

*S. cerevisiae* mitogen-activated protein kinase (MAPK)

*BCK1 MPK1 SWI4 SWI6*

(cell wall integrity pathway)

*STE3 AKR1*

(pheromone signaling pathway)

*STE3 AKR1*

Zymolyase

*AKR1*

*BCK1*

*MPK1*

*FKS2 CHS3*

*CRH1*

30

Calcofluor

white

4

*BCK1*

*MPK1*

*FKS2 CRH1*